EPR Studies Of Rat Intestinal Tissues

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Oxygen radicals and nitric oxide (NO) have been recognized to play a role in the pathophysiology of the intestinal system. In this presentation, EPR spectroscopy will be applied to the intestinal system to study; (1). the interactions of NO and reactive organic radicals generated by *tert*-butyl hydroperoxide (tBOOH), and (2). the metabolism of the intestinal colitis inducer trinitrobenzene sulfonic acid (TNBS). Using a tissue flat cell for direct EPR, we were able to detect *tert*-butyl peroxyl radical (g = 2.013) and TNBS nitro radical anion, when rat intestinal tissues were respectively treated with tBOOH and TNBS. EPR spin-trapping of rat primary intestinal cells treated with tBOOH resulted in the generation of *tert*-butylalkoxyl, methoxyl, and methyl radical adducts. Intestinal epithelial cells are the known source of the inducible form of nitric oxide synthase when rats are challenged with lipopolysaccharide (LPS). Intestinal cells isolated from LPS-treated rats produced decreased levels of tBOOH-derived radicals when these cells were incubated with tBOOH *in vitro*. These decreases in radical production were further decreased when these cells were treated with LPS *in vitro*. These spin-trapping experiments demonstrated that endogenously produced NO could modulate the extent of tBOOH-derived free radical generation by intestinal cells.

For the TNBS studies, EPR spin-trapping experiments demonstrated that diluted suspensions of rat intestinal cells (4-6 X 10^6 cells/ml) or red blood cells (5-10% final concentration), produced TNBS nitro and superoxide radical generation from cellular nitroreductase activity resulting in the redox cycling of TNBS and TNBS radical anion. When intestinal tissues (as scraped mucosa) or red blood cells (25-50% final concentration) were mixed with TNBS, TNBS nitro and sulfite radical were detected. Superoxide radical was not detected under this anaerobic condition. The coupling of C1 of TNBS to the amino group of tissue proteins resulted in the release sulfite that was autooxidized to form sulfite radical. Thus, TNBS metabolism by intestinal cells produced TNBS nitro radical anion, superoxide and sulfite radicals. The latter radicals are precursors of highly reactive radicals, such as, hydroxyl, sulfiteperoxyl and sulfate radicals. These free radicals may contribute to the injury observed in the TNBS-induced colitis *in vivo.*